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NUTRITION REVIEWS

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Nutrition and Tuberculosis 97
Present Knowledge of Vitamin B_6 in Nutrition 98
Dietary Fat and Food Utilization 100
Excess Dietary Calcium in the Rat 102
Clinically Detectable Malnutrition in Italy, 1945 104
Effect of Dietary Protein on Antibody Response 106
Human Requirement of B-Vitamins during Lactation 107
Interaction of Niacin and Tryptophane in the Diet 110
Lymphoid Atrophy and Antibody Production in Pyridoxine Deficiency 111
The Nutritive Values of Proteins Compared with their Content of Essential Amino Acids 112
Nutritional Amblyopia in Prisoners of the Japanese 114
Folic Acid and Pernicious Anemia 115
Choline Requirements and Toxicity 116
Metabolism of Vitamin A and Carotene in Diabetes Mellitus 118
Beriberi and Epidemic Dropsy 119
Biosynthesis of Ascorbic Acid 121
Variation in Human Calcium Requirement 122
Longitudinal Studies of Growth, Development, and Nutriture 124
Notes 127
Letter to the Editor 127
The Copenhagen Conference of FAO 127
Biologic Activity of 3-Methyl-Ascorbic Acid 128
A New Synthesis of Provitamin D_3 128
Nutrition Bulletin 128

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NUTRITION REVIEWS

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NUTRITION AND TUBERCULOSIS

Since the earliest medical writings, nutrition has been considered of prime importance in the treatment of tuberculosis. The medical literature reveals the relationship of nutrition and tuberculosis to be largely on an empirical basis. The history of the treatment of the disease by the use of food is varied and interesting. The early Greek physicians advised woman's milk and the continued use of wet nurses. If the patient would not accept this, the milk of animals still warm with animal heat was offered. Liver, raw eggs, and seafood were also recommended as well as cabbage, various red fruits, and herbs.

Cod liver oil is mentioned often in the list of food substances advised for tuberculous patients. At the beginning of recorded medical history the fisher folk of northern Europe used it externally, for many skin lesions and internally, for wasting diseases. After J. H. Bennett published his treatise on cod liver oil therapy in 1841 (G. B. Webb, "Tuberculosis," *Paul B. Hoeber, Inc., New York (1936)*) it was used rather extensively in England.

The concept that malnutrition predisposes to infectious disease and particularly tuberculosis has been accepted largely on empirical grounds. Well controlled experiments using human subjects have been few. Among the best is the frequently quoted paper of K. Faber (*Ugesk. f. laeger* **101,** *1148 (1939)*) who recounted events in Denmark during World War I when Denmark was selling a high proportion of her foodstuffs, particularly fats, to Germany. At that time the incidence of tuberculosis and xerophthalmia increased greatly. When food could no longer be exported, malnutrition became less evident, xerophthalmia disappeared, and the death rate from tuberculosis dropped.

Earlier in this century a diet largely of milk and eggs was commonly prescribed for the tuberculous patient in the United States. During the same period another practice was also in vogue, namely, overfeeding. The patient was stuffed with food, excessive weight gain being the goal. It is now known that weight gain is not evidence *per se* of retrogression of the disease.

Bed rest and "plenty of good food" are the basis of present day treatment. There is no evidence in the literature that tuberculous persons should be exempt from the recommended dietary for healthy people; on the contrary, there is evidence that the tuberculous person needs even more of some of the food substances. Those treating tuberculosis feel that the patient must have at least the dietary intake recommended for healthy people by the Food and Nutrition Board of the National Research Council, and the diet must be amplified in those food constituents which tuberculous persons seem to need in greater quantities than healthy persons. In some items this becomes a therapeutic problem.

Feeding of the patient on bed rest is a difficult problem for the dietitian, since the appetite often flags and food intake is low. All dietary strategems must be used to maintain adequate food intake.

Objective tests indicate that tuberculous patients may be deficient in ascorbic acid, vitamin A, and protein, and in the advanced stage, calcium (H. R. Getz, I. S. Westfall, and H. J. Henderson, *Am. Rev. Tuberc.* **50,** *96 (1944)*). Ascorbic acid therapy when given alone has proved disappointing (H. C. Sweany, C. L. Clancy, M. H. Radford, and V. Hunter, *J. Am. Med. Assn.* **116,** *469 (1941)*). It is also common knowledge that it is practically impossible to saturate a tuberculous patient with

ascorbic acid if the tuberculous process is progressive. To maintain a blood level approximating that of a normal individual may require that the tuberculous patient receive an ascorbic acid intake of 1000 mg. per day. Treatment with vitamin A has been similarly disappointing and also requires extremely large doses (100,000 I.U. daily) over long periods of time to produce normal blood levels. The deficiencies of ascorbic acid and vitamin A appear to be proportional to the extent of the tuberculous involvement. The needs of the tuberculous patient for the vitamin B-complex remain largely unknown. The low serum albumin is indicative of protein deficiency which had been overlooked for years because the total serum protein is usually normal in these patients.

Present knowledge dictates a diet adequate in calories to restore normal weight. Return to normal weight need not be rapid and can usually be accomplished with a diet containing 2500 to 3000 calories. A fairly high fat intake is needed to get energy to the patient in little bulk. Most clinicians with experience in treating tuberculosis feel that tuberculous persons need more protein than healthy persons. As knowledge of amino acid requirements in disease is extended the high protein diets advocated by F. M. Pottenger, Jr. and F. M. Pottenger (*Am. Rev. Tuberc.* **54,** *213 (1946)*) may be indicated. There has been much controversy concerning the role of minerals in the therapy of tuberculosis. Calcium rich foods are indicated in the advanced case which has a lowered serum calcium and in extreme cases supplements of calcium are indicated. Iron is needed for hemoglobin regeneration especially by those patients suffering large or repeated hemorrhages.

Nutritional deficiencies in the tuberculous patient are undoubtedly multiple and are not clearly understood. The need for additional ascorbic acid and vitamin A seems to be well established. In almost any sanatorium with large numbers of far advanced cases of tuberculosis there are patients with evidence of scurvy and some with all the known signs of vitamin A deficiency. The exact significance of these observations is unknown. However, since these nutritional deficiencies have been found, it seems wise to recommend a diet and vitamin therapy aimed at their abolition.

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PRESENT KNOWLEDGE OF VITAMIN B_6 IN NUTRITION

The existence of an essential vitamin for rats distinct from vitamins B_1 and B_2, which was called vitamin B_6, was proved by György about 1934. A crystalline compound active in curing symptoms of vitamin B_6 deficiency was isolated almost simultaneously in five different laboratories in 1938, and synthesized in two laboratories in 1939. The name "pyridoxine" was adopted for this substance. "Pyridoxine" and "vitamin B_6" then became synonymous terms. Later, however, rats and human beings were shown to convert pyridoxine to one or more substances which far surpass pyridoxine in growth-promoting potency for lactic acid bacteria (E. E. Snell, B. M. Guirard, and R. J. Williams, *J. Biol. Chem.* **143,** *519 (1942)*). A similar substance occurs in tissue extracts, and was called "pseudopyridoxine." Subsequent study led to synthesis of two compounds, pyridoxamine and pyridoxal, which showed the high activity for lactic acid bacteria predicted for "pseudopyridoxine" and are also active as vitamin B_6 for animals (*Nutrition Reviews* **3,** *63, 327 (1945)*). Since both of these compounds occur naturally (*Ibid.* **3,** *327, 343 (1945)*), the term "vitamin B_6"

when applied to the naturally occurring substance, refers to a group of at least three related compounds, and possibly others of unknown nature (*Ibid.* **4,** *111* (*1946*)).

The determination of vitamin B_6 in natural products is complicated by the coexistence of these three compounds, together with bound forms such as phosphorylated pyridoxal (*Nutrition Reviews* **3,** *72, 343* (*1945*); **4,** *232* (*1946*)) and a conjugate of pyridoxine which constitutes a major portion of the vitamin present in rice bran extracts (J. V. Scudi, *J. Biol. Chem.* **145,** *637* (*1942*)). Present methods for determining vitamin B_6 fall into three categories: (1) chemical procedures, depending upon coupling of the vitamin with 2,6-dichloroquinone chlorimide or with diazotized sulfanilic acid to produce colored substances; (2) biologic assay with rats, and (3) microbiologic procedures. Chemical procedures were developed before the existence of multiple forms of the vitamin was recognized; their specificity when applied to foodstuffs needs reinvestigation. Assays with rats, remain, of course, the primary standard. It is of interest, therefore, that pyridoxal and pyridoxamine may show activity equal to, or less than, that of pyridoxine (P. S. Sarma, E. E. Snell, and C. A. Elvehjem, *J. Biol. Chem.* **165,** *55* (*1946*)). If injected, or administered by dropper apart from other food, the three compounds are equally active; if fed mixed with the ration, pyridoxal and pyridoxamine are somewhat less active than pyridoxine. The latter procedure, obviously, simulates most closely the situation which exists normally.

A microbiologic method utilizing yeast (L. Atkin, A. S. Schultz, W. L. Williams, and C. N. Frey, *Ind. Eng. Chem.* (*Anal. Ed.*) **15,** *141* (*1943*)) has been used widely; the method is useful since the test organism responds equally to the three known forms of the vitamin. Other microorganisms are known which utilize only pyridoxal, or pyridoxal plus pyridoxamine (*Nutrition Reviews* **3,** *327* (*1945*)).

Availability of pyridoxal and pyridoxamine has permitted rapid progress in elucidation of metabolic functions of vitamin B_6. Phosphorylated pyridoxal serves as an essential prosthetic group (codecarboxylase) of enzymes which decarboxylate tyrosine, arginine, glutamic acid, "dopa," and other amino acids (*Nutrition Reviews* **3,** *343* (*1945*); **4,** *232* (*1946*); E. F. Gale, *Advances in Enzymology* **6,** *1* (*1946*)), and also for enzymes which carry on transamination reactions (*Nutrition Reviews* **4,** *232* (*1946*)). In at least some cases a phosphorylated pyridoxamine can also function with the latter enzymes (W. W. Umbreit, D. J. O'Kane, and I. C. Gunsalus, *J. Bact.* **51,** *576* (*1946*); F. Schlenk and A. Fisher, *Arch. Biochem.* **12,** *69* (*1947*)). In accordance with these observations, tissues from vitamin B_6 deficient rats show lowered capacity for transamination (*Nutrition Reviews* **4,** *232* (*1946*)) and a lowered concentration of codecarboxylase (W. D. Bellamy, W. W. Umbreit, and I. C. Gunsalus, *J. Biol. Chem.* **160,** *461* (*1945*)). These observations provide, in part, a chemical basis for earlier observations that pyridoxine was involved in protein metabolism. It is not clear, however, how vitamin B_6 enters into the metabolism of tryptophane. This amino acid is partially excreted as xanthurenic acid by pyridoxine deficient rats, dogs, and swine (*Nutrition Reviews* **3,** *72* (*1945*); **4,** *150* (*1946*)), and as a yellow pigment of unknown composition by deficient rice moth larvae (*Ibid.* **3,** *173* (*1945*)). With adequate vitamin B_6 in the ration, these excretion products do not appear. Pyridoxine also alleviates toxic symptoms produced in rats receiving dl-serine by stomach tube (W. H. Fishman and C. Artom), *Proc. Soc. Exp. Biol. Med.* **57,** *241* (*1944*)). Dietary requirements of vitamin B_6 for mice are increased by high protein rations (E. C. Miller and C. A. Baumann, *J. Biol. Chem.* **157,** *551* (*1945*)). All of these

results point to the importance of this vitamin in protein metabolism. Less clearcut evidence indicates that it is also involved in fat metabolism (e.g., H. Schneider, H. Steenbock, and B. R. Platz, *Ibid.* **132,** *539 (1940)*).

Vitamin B_6 is required in the diet of all animals so far investigated. Aside from impaired growth, which is shown by all immature animals held on a vitamin B_6 free diet, deficient rats may show an acrodynia associated with seborrheic lesions (*Nutrition Reviews* **1,** *157 (1943)*). Edema of the connective tissue layer of the skin (the corium) (*Ibid.* **2,** *282 (1944)*), convulsive or epileptoid seizures, and muscular weakness (*Ibid.* **1,** *26 (1942)*; **2,** *261 (1944)*) are characteristic.

Vitamin B_6 deficient dogs and swine develop a severe microcytic, hypochromic anemia (*Nutrition Reviews* **3,** *29, 343 (1945)*), accompanied by a two-to fourfold increase in the level of plasma iron, by hemosiderosis in the liver, spleen, and bone marrow, and by hyperplasia of the bone marrow. Despite high plasma iron and hemosiderosis, the anemia is not of the hemolytic type, since the icterus index, serum bilirubin, and porphyrin excretion are not increased. Furthermore, if the diet is also low in iron, plasma iron levels remain low, and hemosiderosis does not occur. Both species may also exhibit epileptoid convulsions. After nine to ten weeks on the deficient ration, demyelinization of the peripheral nerves and degeneration of the axial cylinder can be demonstrated in swine (*Ibid.* **3,** *269 (1945)*). Cattle on suitably restricted rations show anorexia, poor growth, unthriftiness, poor condition of the hair coat, and poikilocytosis. The condition is cured by administration of pyridoxine (*Ibid.* **4,** *186 (1945)*).

It is natural that pyridoxine should be tried clinically in conditions showing points of similarity to symptoms of deficiency in animals. Therapeutic trials in patients with muscular weakness, epilepsy, certain types of dermatitis, and with nausea and vomiting of pregnancy (*Nutrition Reviews* **4,** *163, 170 (1946)*) have been made. In only a few cases have positive results been reported. No human counterpart of the animal anemias produced by vitamin B_6 deficiency has been recognized. Much more work, of a carefully controlled nature, is required to evaluate the usefulness of this vitamin in medical practice.

DIETARY FAT AND FOOD UTILIZATION

Voit characterized the ideal food as a well-tasting mixture of foodstuffs arranged together in such proportion as to burden the organisms with a minimum of labor. The search for the most efficient arrangement of nutrients during the sixty-five years which have passed since this statement was made has yielded amazing data that certainly were not part of Voit's original conception. Nevertheless, the goal has not yet been reached. The incompleteness of our knowledge concerning the finer details of the interrelationship of nutrients is illustrated by the current uncertainties regarding the proper type and proportion of fat in human and animal dietaries. The relative nutrient values of animal and vegetable fats (*Nutrition Reviews* **4,** *140 (1946)*), the effect of thiamine in fat metabolism (*Ibid.* **1,** *58 (1942)*; **2,** *308 (1944)*), the influence of different fats on tumor growth (*Ibid.* **2,** *146, 338 (1944)*; **3,** *219 (1945)*), and the economic aspect of the fat content of dairy feeds (*Ibid.* **2,** *213 (1944)*) are a few of the problems which require further investigation.

The belief that fat and carbohydrate are interchangeable in the diet according

to their metabolizable energy values, the isodynamic law of Rubner, has been accepted as a fundamental fact. Until recently, the chief qualifications have involved extraneous factors, such as the role of fats as carriers of vitamins, sterols, and other fat soluble compounds, and the effect on the palatability of food. An additional limitation on the extent of substitution of fat for carbohydrate is based upon the relative rates of production of acetoacetic acid in the liver (*Nutrition Reviews* **4,** *23 (1946)*) and the rate of oxidation of this substance in all tissues of the body. A loss of metabolizable energy from fat occurs if ketonuria results from excessive ketogenesis. A further qualification of the isodynamic law is indicated by the observation of E. B. Forbes and R. W. Swift (*Ibid.* **3,** *82 (1945)*) that the partial replacement of carbohydrate by fat in a mixed dietary containing protein diminishes the energy expenditure of utilization of food (the specific dynamic effect) and results in a sparing of energy which otherwise would have been wasted. In other words, animals have extra calories for growth, work, or storage on a mixed diet containing fat, calories which are not available in the absence of fat even though an isocaloric mixture of carbohydrate and protein is supplied.

The study of this highly significant property of dietary fat has been continued by E. B. Forbes, R. W. Swift, R. F. Elliott, and W. H. James (*J. Nutrition* **31,** *203, 213 (1946)*). Strictly comparable groups of young rats were fed isocaloric diets containing 2, 5, 10, and 30 per cent of fat. The four diets were so prepared that each provided equal amounts of gross energy, protein, and vitamins. Gains in weight, in nitrogen, and in energy were measured after a seventy day period. Complete urinary and fecal collections were made and the total heat production was determined by subtracting the energy value of the excreta and the energy value of the body gain from the energy value of the metabolizable food. The gains in live weight, the digestibility of protein, and the retention of nitrogen and of energy were found to be in the order of the increasing fat content of the diets. The superiority of the 5 per cent over the 2 per cent fat diet was much greater than the superiority of the 30 per cent over the 10 per cent fat diet. The extent of the sparing effect of fat on the utilization of food is indicated by the fact that the retention of nitrogen as tissue protein and of energy as tissue protein and fat were 10 and 13 per cent greater, respectively, on the 30 per cent fat diet than on the diet containing 2 per cent of fat.

The effect of fat on the efficiency of utilization of food was also measured in adult rats fed the isocaloric diets containing 2, 5, 10, and 30 per cent of fat at two levels, a maintenance level (44.6 calories daily) and a supermaintenance level (64.9 calories daily). Respiratory measurements were made in addition to determinations of the energy content of the food and of the excreta. The energy expense of utilization of food (the specific dynamic effect) was measured as the difference between the heat production on the two nutritional levels. The waste of energy due to the specific dynamic effect of the extra 20 calories of food, supplied as a mixture of protein, carbohydrate, and fat in the supermaintenance diet, decreased from 36 to 20 per cent if the fat content of the diet was increased from 2 to 30 per cent. The net effect was the same as would be expected if the rats on the isocaloric but higher fat diet had actually received an additional 3 calories daily.

More recently, E. B. Forbes *et al.* (*J. Nutrition* **32,** *387, 397 (1946)*) have repeated the studies on growing and mature rats, respectively, to determine if larger supplements of vitamins would affect the results. This second investigation was in general agreement with the previous study.

The authors conclude from these experi-

ments that fat confers efficiency of utilization of food energy in both growing and mature rats. The principal saving resulted from the depression of the specific dynamic action of the food. It was evident that the effect of fat is not limited to better utilization of fat alone, because the retention of nitrogen in the growing rats was increased. The data do not conflict with the idea that carbohydrate is used more efficiently than fat for work because all of the diets contained sufficient carbohydrate. In this connection, the authors point out that their results might be ascribed to the effect of decreasing the carbohydrate or of increasing the fat of the diet. The practical significance of the observations is the same regardless of the method of expression.

These investigations by Forbes *et al.* are excellent illustrations of experimentation in which a combination of several laboratory methods is utilized, including measurements of the respiratory exchange, of nitrogen balance, of growth, of change in composition of tissues, and of the over-all energy balance. The data should be of value in the interpretation of hitherto unexplained effects of various nutrients in diets of different fat content. From the economic viewpoint, re-evaluation of the practice of feeding low fat rations to livestock would appear desirable. The studies provide an additional and important illustration of the beneficial effect of "proper balance" of nutrients in nutrition.

EXCESS DIETARY CALCIUM IN THE RAT

Various relationships of nutrition to atabrine toxicity have been demonstrated (*Nutrition Reviews* **2,** *207* (*1944*)). For example, rats on a high-protein, low-fat diet have been found to resist the toxic effects of atabrine to a greater extent than rats maintained on a low-protein diet or on one high in both protein and fat. In studies of single nutritional deficiencies, it has been shown that the slow rate of growth obtained with diets containing suboptimal amounts of riboflavin or protein was further decreased by the addition of atabrine to the diets. With diets suboptimal in vitamin A, this effect was not observed. In addition atabrine has been found to protect rats against the development of kidney hemorrhages on a choline deficient regimen. Atabrine has also been shown to have a thiamine-sparing action in the young rat (D. M. Hegsted, J. M. McKibbin, and F. J. Stare, *J. Nutrition* **29,** *361* (*1945*)).

In a continuation of these studies, the effect of variations in the level of dietary calcium upon the growth of young rats receiving atabrine has been investigated by A. Williamson, D. M. Hegsted, J. M. McKibbin, and F. J. Stare (*J. Nutrition* **31,** *647* (*1946*)). Diets which contained 0.15, 0.55, and 1.35 per cent calcium, respectively, but which were otherwise identical were used for these studies. Rats which received 40 or 60 mg. per cent atabrine with the high calcium diet attained only 51 to 63 per cent of the weight gain of their respective controls which did not receive atabrine. In comparison, rats which received the same atabrine supplements to the lower calcium diets grew 67 to 77 per cent as well as their controls. This difference was found to be statistically significant. The amount of atabrine consumed by the rats on the different levels of calcium was in the neighborhood of 4 mg. per day. Despite this fact, the atabrine concentration in the livers and spleens of the rats fed the high calcium diet was considerably higher than the atabrine concentration in the livers and spleens of the rats fed the lower levels of calcium. The atabrine concentration

was not dependent upon the duration of atabrine feeding since the concentration in the livers of rats receiving either the high or low calcium levels was no higher after seven weeks than after half that time. The high levels of atabrine observed in the liver and spleen of the rats maintained on the high calcium diet may have been influenced by a pathologic condition of the kidneys. In several animals on the high calcium diet without an atabrine supplement, the kidneys were hypertrophied with numerous stones.

Experiments were then conducted to determine if the degree of atabrine toxicity might be altered by the acidity or alkalinity of the intestinal contents. Since a high amount of calcium in a purified diet results in a more alkaline intestinal reaction, this was an attempt to determine whether a more alkaline intestinal reaction had been responsible for the greater atabrine toxicity observed on the high calcium diet. Various materials were tested but only magnesium carbonate and the calcium carbonate of the high calcium diet resulted in an increased alkalinity of the intestinal contents. This change was apparent only in the lower portion of the small intestine and in the cecum. The presence of atabrine in the rations did not alter the reaction of the intestinal contents. Citric acid and ammonium salt supplements to these rations did not alter the reaction of the intestinal contents but did alter different manifestations of the atabrine toxicity. Citric acid improved the weight gains of the rats on the high calcium ration plus atabrine but had no effect on the weight gains of the rats maintained on the low calcium diet. On the other hand ammonium salts did not affect the response to the high calcium diet but did increase the growth inhibition due to atabrine on the low calcium diet.

Little is known of the effect of high calcium diets on the calcium content of the soft tissues, especially in adult animals. J. B. Shields and H. H. Mitchell (*J. Nutrition* **32**, *213 (1946)*) have reviewed the literature pertaining to the various changes in tissue calcium which have been observed in senescence and in certain diseases. To study whether a high-calcium diet, especially in adult life, would affect the occurrence of soft tissue calcification and the life expectancy, 24 trios of albino rats were fed from shortly after weaning until natural death on three experimental diets which contained liberal amounts of all known essential nutrients and differed only in calcium and phosphorus contents. During the first forty-three weeks of feeding, diet A consisted of the basal ration with enough calcium carbonate to raise the calcium content to approximately 0.6 per cent. At the end of forty-three weeks, the calcium content was reduced to 0.2 per cent. Diets B and C included supplements of dicalcium phosphate to raise the calcium content to approximately 1.0 per cent. At the end of forty-three weeks, the calcium content of diet B was reduced to 0.7 per cent, while diet C was unchanged.

The rats in all diet groups increased in body size at a satisfactory rate with no significant difference in the rates of growth among the rats maintained on the three diets. A great variation was observed in the longevity of the rats in all three groups. A statistical analysis of this variation indicated that the random distribution of the uncontrolled factors in the experiment would have resulted in this variation without the influence of the calcium variation of the diets. There was a slight tendency towards higher calcification in the tissues of the rats which were maintained on the high level of calcium throughout life. The complications accompanying terminal illness of these rats made exact interpretation of the differences impossible. The data obtained in this experiment tended to indicate that the accumulation of calcium in the soft tissues was more dependent upon the local conditions than upon systemic ones.

In view of the essentially negative observations of Shields and Mitchell, the studies

of Williamson *et al.* with growing rats maintained on an even higher calcium diet are interesting for comparative purposes. In both investigations, no evidence of slower growth was observed in the rats maintained on the high calcium diets. However in the latter experiment, definite lesions were observed in the kidneys of the rats on the high calcium diets. In addition the supplements of atabrine to the high calcium diet had a greater growth retarding effect than when the same supplements of atabrine were added to moderate or low calcium diets. It was not stated whether the lesions in the kidneys were more extensive or more severe when atabrine was added to the high calcium diet. In the growth data, there was a definite interrelationship between the calcium content of the diet and the atabrine supplements. Thus in the normal animal there may be an ability to excrete calcium ingested far in excess of the normal requirements; under conditions of stress this ability may decrease. A study of the calcium content of the tissues as performed by Shields and Mitchell using atabrine-fed rats might supply some interesting information on this postulation.

CLINICALLY DETECTABLE MALNUTRITION IN ITALY, 1945

Many of the nutrition surveys carried out in the United States have been aimed at sampling a true cross section of the population. A report by J. Metcoff and A. J. McQueeney (*New. Engl. J. Med.* **235,** *451 (1946)*) illustrates another method of sampling which directs attention toward various stress groups within the population; namely, children and pregnant and lactating women. The deliberate selection of these groups for study is made in an effort to obtain the worst possible picture existing within the population. For such purposes, this procedure appears to have served a useful purpose. It is well to recognize, however, that these particular categories are the groups who are allowed additional rations in the usual rationing programs. Hence, without some check on the validity of the basic assumption that the groups do, in practice, represent the most malnourished group of the population, this method of sampling could lead to error.

Metcoff and McQueeney have here summarized the results of 35 rapid surveys involving approximately 4000 individuals selected from refugee, institutional, and school groups, and from the population at large. The population was located in urban and rural areas in northern, central, and a portion of southern Italy.

The examination procedure consisted of a rapid inspection for signs of deficiency diseases. The inspection included the hands, arms, legs, thorax, eyes, nose, lips, teeth, gums, tongue, ears, neck, and skeletal structures. A limited neurologic examination was carried out. Sahli hemoglobin determinations were made on a portion of the individuals, usually on alternate subjects. Plasma protein estimations were occasionally made.

The clinical criteria adopted by these investigators were well defined and were restricted to classic deficiency manifestations. For a diagnosis of rickets, the subject must have presented at least three skeletal signs of the disease. The diagnosis of ariboflavinosis was made on the presence of angular stomatitis associated with denudation with or without fissuring of the lower lip, glossitis, and sometimes nasomalar seborrheic dermatitis. The diagnosis of vitamin A deficiency was restricted to those subjects presenting typical keratosis follicularis and conjunctival changes excluding pterygiums and pingueculas. Inanition was used to describe cases with obvious loss of

subcutaneous tissue. Anemia was defined as a hemoglobin level of less than 8.0 g. per 100 ml. in infants under 2 years of age and less than 10.0 g. per 100 ml. in children and adults. Nonspecific glossitis was noted and described but was not interpreted in relation to specific deficiency disease. Gingivitis, frequently encountered, was not considered to be of scorbutic origin unless associated with other findings. Pellagra, beriberi, scurvy, or hypoproteinemia were not encountered.

The most frequently observed deficiency disease was rickets, the incidence in particular surveys ranging from 25 to 30 per cent among children to 60 per cent among younger children and institutionalized infants. The over-all incidence was 27.7 per cent. Clinical cases of ariboflavinosis were rarely encountered, the incidence varying from 0 to 2 per cent. The general incidence of clinical vitamin A deficiency was estimated at 1.5 per cent. Inanition was most commonly encountered in the institutionalized children, but here was often independent of the available food supply. Again, the institutionalized infants and preschool children showed the highest incidence of moderately severe anemia. Only about 0.5 per cent of the schoolchildren were classified as anemic and but 1.5 per cent of the pregnant and lactating women. Thyroid enlargement was observed in from 5 to 9 per cent of the children and 18 to 23 per cent of the pregnant and nursing women. The apparent indifference in Italy to this nutritional problem is striking when it is recalled that iodine-deficiency goiter has been demonstrated as an easily preventable disease and that the endemicity of goiter in Italy has long been recognized (see C. A. Ewald, "Die Erkrankungen der Schilddrüse, Myxödem und Cretinismus, in Specielle Pathologie und Therapie," **22,** *Vienna* (*1896*)). In summary, rickets appeared to be the only clinically manifest deficiency disease which was classified as presenting a public health problem. One might also add thyroid enlargement in view of these data. Grossly evident clinical malnutrition was not considered to be serious enough in degree or sufficiently wide in distribution to constitute an acute relief feeding problem. The high incidence of clinical rickets did not appear to be directly attributable to the short supplies of cod liver oil and vitamin D concentrates. The authors note that there existed numerous hospitals for the treatment of rachitic deformities in children, suggesting that clinical rickets had been a widespread problem in Italy prior to the war. Endemic dental fluorosis in Campagna da Roma was almost universal. Again, this was not a relief problem.

When the height-weight data for the various ages were plotted on charts indicating the 80 percentile ranges for height and weight for normal American children (V. S. Vickers and H. C. Stuart, *J. Pediatrics* **22,** *155* (*1943*)) it was apparent that these measurements fell for the most part within the lower limits of the range. These findings must be interpreted with due regard for the differences in sampling technics employed between the Italian and American groups. The investigators state that their observations suggest "no marked deviation in the general population from pre-war Italian standards for pre-school and school-age children." In summary, it seems probable then that except for rickets and, perhaps, some limitation of growth and suggestive thyroid enlargement, no conclusive evidence could be obtained for the existence of deficiency states. Furthermore, the wartime food shortages apparently instituted no major health problem in the populations studied.

The similarity of this report with the conclusions of an earlier review of the nutritional state of children in France (H. C. Stuart, *Am. J. Pub. Health* **35,** *299* (*1945*)) is most striking. In this earlier review, Stuart observed that "the only striking and serious vitamin deficiency encountered in the studies was lack of vitamin D." Stuart

did not attribute this high incidence of rickets among the infants entirely to the effect of the war; rather, he noted that physicians in France generally did not employ antirachitic substances as preventive measures as widely as is the custom in the United States. He reported that 50 per cent of all those children examined between the ages of 6 and 18 months showed signs of early rickets. Again, Stuart noted that the most remarkable feature of the measurements on the French children compared with those of American children was their very short stature for age. This he attributed to dietary faults antedating the war, including inadequate calories and animal protein. Lack of vitamin D was also considered a factor in the stature difference. In 1943, 15 per cent of the French children observed had hemoglobin levels under 10.0 g. The origin of this anemia was undetermined. Stuart observed further that the clinical deficiencies of vitamin A were "very infrequent and mild," that no clearcut clinical evidences of iron deficiency were found, but that "a number" of cases exhibited signs suggesting riboflavin deficiency. He found no clinical or x-ray evidence of ascorbic acid deficiency.

These two reports seem to justify two major conclusions. First, that antirachitic substances should be used more widely in these areas than they have been in the past, even before the restrictions due to the war developed. Secondly, that in times of food shortages growing children are more likely to evidence caloric and protein deficiency than they are to develop clearly recognizable vitamin deficiencies under the dietary habits which prevailed in these two countries.

EFFECT OF DIETARY PROTEIN ON ANTIBODY RESPONSE

Increased susceptibility to infection generally accompanies poor states of nutrition. Death rates from tuberculosis and some other infectious diseases increase in periods of famine. The manner in which malnutrition is effective in decreasing resistance to infection has not been fully ascertained. However, the experiments of P. R. Cannon and his collaborators (*Nutrition Reviews* **1,** *186 (1943)*; **2,** *197 (1944)*; **3,** *78 (1945)*) have demonstrated that antibody production is decreased in protein-depleted rabbits and rats. It is postulated that this is the result of an inadequate supply of protein precursors necessary for the synthesis of gamma-globulin, the antibody protein. In recent reports these workers (R. W. Wissler, R. L. Woolridge, C. H. Steffee, and P. R. Cannon, *J. Immunology* **52,** *267 (1946)*; R. W. Wissler, R. L. Woolridge, and C. H. Steffee, *Proc. Soc Exp. Biol. Med.* **62,** *199 (1946)*) have provided further evidence to support this hypothesis by showing that the addition of high-quality proteins, an enzymatic digest of casein, or a mixture of crystalline amino acids to the diet of rats previously depleted of protein, brings about a prompt increase in the capacity of the animals to produce antibody.

In these experiments protein depletion was accomplished by maintaining adult male rats on a diet which contained approximately 2 per cent protein but which was assumed to be adequate in other respects. Depletion of protein stores was gradual and extended over a period of from three to six months. There accrued a loss of weight, a decrease in serum proteins, and a fall in hemoglobin content of the blood. A week before the injections of the antigen (sheep red blood cells) the prepared rats were placed on a diet completely devoid of protein. Antibody (hemoglobin) titers were subsequently determined at intervals of four, six, ten, and fifteen days after the injection. The average maximum titer of antibody in this group of rats was only one sixth of that attained by a group which had

not been previously depleted of protein and the diets of which at all times contained adequate protein. When dehydrated beef in quantities providing 20 per cent of the diet as protein was given to rats which had been previously depleted of protein, the response in antibody production was demonstrably increased after only two days of protein realimentation and was increased further if an interval of seven days on the beef protein diet was allowed before the injection of antigen. The provision of 22 per cent casein or 11.8 per cent lactalbumin in the diet during the realimentation regimen brought about a similar increase in antibody production. An enzymatic hydrolysate of casein (20 per cent of the diet) and a mixture of 16 crystalline amino acids modeled after the amino acid composition of casein were similarly effective in restoring antibody production. In each instance the increase in antibody response was accompanied by an increase in body weight, serum protein, and hemoglobin concentrations. Administration of a liver concentrate or of 5 mg. of ascorbic acid per day did not influence the antibody response.

It must be considered significant that antibody production in rats depressed by maintaining the animals on low protein diets for periods as long as six months is quickly restored by addition to the diet of adequate quantities of high quality protein or of properly selected amino acid mixtures. Indications are that this is the result of replenished protein reserves permitting an increased rate of synthesis of gamma-globulin. This would seem to be a process of general importance in systems of antibody response in higher animals, since information available at the present time indicates that systems of protein synthesis show little variation from species to species. It could hardly account for the widespread variation in antibody response to the same antigens in different species. It should be recognized also that antibody production is only a part of the total response of the organism to infection and that other factors undoubtedly are effective in determining susceptibility or resistance. In considering the work from Cannon's laboratory one should always bear in mind that the experimentally induced antiresponse is not to an infectious agent but to a foreign protein—sheep red blood cells.

There is in medical annals a classic instance in which an adequate dietary was shown to be an important adjunct in the treatment of an infectious process. At the turn of the century a starvation regimen was an important part of the treatment of typhoid fever. When this concept was abandoned and attempts were made to provide an adequate diet including large quantities of protein, the mortality rate for the disease decreased rather remarkably.

HUMAN REQUIREMENT OF B-VITAMINS DURING LACTATION

Recommended allowances of thiamine and riboflavin for women during lactation are 2 and 3 mg., respectively, on a 3000 calory basis, or 0.67 and 1.0 mg. per 1000 calories (*Nutrition Reviews* **3,** *287* (*1945*); **4,** *130* (*1946*)). These values represent intakes which are intended to provide a reasonable factor of safety. The exact minimum requirements are uncertain and can be ascertained only by the accumulation of sufficient data on the relation of the dietary supply of thiamine and riboflavin to infant and maternal health (*Ibid.* **3,** *248* (*1945*); **4,** *134*, *175* (*1946*)). Studies of this type on the utilization of thiamine during lactation have been reported by C. Roderuck, H. H. Williams, and I. G. Macy (*J. Nutrition* **32,** *249* (*1946*)) and on the utilization of riboflavin by C. Roderuck, M. N. Coryell, H. H. Williams, and I. G. Macy (*Ibid.* **32,**

267 (1946)). The subjects were 14 women, 7 of whom were studied during the first ten days postpartum and 10 during a total of eighteen five day periods at various intervals throughout the later months of lactation. Analyses were made of duplicate meals, of twenty-four hour collections of milk and urine, and of fasting one hour samples of urine. The latter were taken at the start and end of the five day periods, twelve to thirteen hours following the last meal. The thiamine intake ranged from 0.73 to 1.59 mg. daily and that of riboflavin from 2.2 to 3.6 mg. The diets consisted of natural foods and were believed to be adequate in other nutrients. Liver was fed on the fifth day of each period, the extra riboflavin from this source amounting to 50 per cent more than the mean intake of the previous four days.

The volumes of milk secreted from the sixth to the tenth days were more than double the amounts produced during the first five days postpartum and were larger than volumes obtained from the same women during later stages of lactation. There appeared to be no relation between the volume of milk and the vitamin intake. The small volume of milk secreted on the first day was very low in thiamine (0.0015 mg. per 100 ml.) and in riboflavin (0.02 mg. per 100 ml.). The total twenty-four hour secretions on the first, fifth, and tenth days were 0.001, 0.023, and 0.095 mg. of thiamine and 0.01, 0.35, and 0.49 mg. of riboflavin. The average concentration of riboflavin per 100 ml. of milk reached a maximum of 0.035 mg. on the fifth day but that of thiamine was still considerably below the maximum (0.015 mg. per 100 ml.) on the tenth day, at which time the average value was only 0.008 mg. Thiamine and riboflavin occurred in milk in free and combined forms, the combined forms averaging 56 and 30 per cent, respectively, of the totals.

Both volume of milk and content of vitamins varied widely for the different subjects but the concentration was relatively constant in each subject after the attainment of the maximum value. For instance, the average levels of thiamine in one case were 0.002, 0.005, 0.015, 0.012, 0.013, and 0.016 mg. per 100 ml. of milk collected during five day periods after one, six, seventy-eight, one hundred and sixty-one, two hundred and thirty-nine, and three hundred and two days postpartum. The corresponding total secretions were 0.014, 0.086, 0.126, 0.112, 0.089, and 0.062 mg. These quantities represented 2 to 12 per cent of the intake (daily average, 1.11 mg.). The riboflavin values for the same periods were as follows: concentration per 100 ml. of milk, 0.033, 0.040, 0.048, 0.042, 0.043, and 0.046 mg.; total secretion, 0.26, 0.72, 0.41, 0.38, 0.29, 0.18 mg.; and, per cent of intake (daily average, 2.95 mg.), 6 to 32. The maximum daily secretion of thiamine in milk never exceeded 15 per cent of the intake, either in this subject or in the others. The urinary excretion ranged from 13 to 28 per cent of the intake for this subject and from 2 to 57 per cent for all subjects. Because no indication of thiamine avitaminosis was observed in these experiments, it was suggested that a 15 per cent increase in the dietary supply over that of the minimum requirement of adult normal women may be sufficient for the extra demands of lactation. In arriving at this conclusion it is necessary to assume that there is no wastage of thiamine in the production of milk by mammary tissue. The authors noted that the largest percentages of thiamine "unaccounted for" in milk and urine occurred in the five day period following parturition and proposed the possibility that muscle metabolism during labor may have depleted the maternal stores of the vitamin and necessitated replenishment from the dietary supply. As a matter of fact, these per cent values are higher immediately after parturition because the intake of thiamine at this time was low. The average differences between intake and elimination were 0.87 mg. for the first five days postpartum, 0.88 mg. for the sixth to the tenth day, 0.89 mg. for five

day periods between forty-five and one hundred days, and 0.92 mg. for five day periods after one hundred days. If the "unaccounted for" thiamine represents utilized vitamin, the fact that this portion was smallest immediately after parturition raises the question of the adequacy of the thiamine intake during this period. However, the average urinary excretion was not less than previously reported values for normal subjects. Furthermore, fasting one hour urine samples exceeded the value of 0.0006 mg. suggested by R. E. Johnson, F. Sargent, P. F. Robinson, and F. C. Consolazio (*War Medicine* **7,** *227 (1945)*), as the lower limit of excretion on satisfactory thiamine intakes.

The average daily secretion of riboflavin in milk in all five day periods was 9 per cent and the excretion in urine was 47 per cent of the intake, which was approximately 3 mg. The "unaccounted for" riboflavin amounted to 1.5 mg. daily in all periods except the second five day period postpartum when the value averaged 0.56 mg. This was undoubtedly due to the elimination of riboflavin in milk and in urine being greatest during this interval. The significance of this observation is obscured by the fact that the period in question was the only one which followed immediately after the ingestion of extra riboflavin in the form of liver. Despite this complication, it appeared that a greater wastage in the form of urinary riboflavin occurred during the period of maximum secretion of milk and of riboflavin in milk. That the subjects were in negative nitrogen balance six to ten days postpartum may be of possible significance. At all levels of mature milk secretion the average ratio between thiamine and riboflavin was approximately 0.4, the same ratio as the average intakes of the two vitamins.

Fasting one hour urines collected on the first day of the five day periods contained from 0.014 to 0.054 mg. of riboflavin with an average of 0.030 mg. Four of twelve samples from 5 subjects contained less than 0.020 mg., the level considered by Johnson *et al.*, as representing the minimum excretion on satisfactory riboflavin intakes. These determinations were repeated at the end of the period, twelve hours after the meal containing liver. At this time the one hour excretion ranged from 0.025 to 0.072 mg. with an average of 0.053 mg. In another subject who had been taking a riboflavin supplement the initial fasting one hour excretion was 0.154 mg.

These studies, which are a part of an extensive investigation of the metabolism of women during the reproductive cycle, indicate that satisfactory lactation is possible on diets supplying approximately 1 mg. of thiamine and 3.0 mg. of riboflavin daily during the first ten days after parturition, the second half of this period representing the phase of maximum milk and riboflavin secretion. The small amount of thiamine in the milk at this period is extremely interesting and one is tempted to associate it with the low thiamine intake. Such a conclusion is not supported by the data on the one subject who was known to have taken a daily vitamin supplement, including 3.6 mg. of thiamine, throughout pregnancy. The immature milk of this individual was not characterized by an increased level of thiamine.

The secretion of thiamine and riboflavin in milk averaged less than 10 per cent of the intake during the first ten days postpartum as well as during all subsequent periods. This might indicate that the minimum daily requirement of normal women is not more than 0.9 mg. of thiamine and 2.7 mg. of riboflavin. The thiamine value is slightly lower than the 1 mg. intake suggested by earlier reports and more recently by H. G. Oldham, M. V. Davis, and L. J. Roberts (*J. Nutrition* **32,** *163 (1946)*) for young women. Although this level appeared adequate for lactation in the studies of Roderuck *et al.*, it should be emphasized that data were not available on the dietary thiamine prior to parturition and lactation except for one subject. The relatively large

excretion of urinary riboflavin in these subjects indicates that the intake of 3.0 mg. daily was in excess of the minimum requirement for lactation. However, the fate of "unaccounted for" thiamine and riboflavin needs clarification before definite conclusions are possible concerning minimum requirements of these vitamins.

INTERACTION OF NIACIN AND TRYPTOPHANE IN THE DIET

The effect of different proteins in the diet upon growth failure in rats receiving a considerable proportion of corn products has been well established (*Nutrition Reviews* **4,** *233 (1946)*). W. A. Krehl, J. de la Huerga, and C. A. Elvehjem (*J. Biol. Chem.* **164,** *551 (1946)*) have shown that niacin exerts an important influence on the utilization of the tryptophane in diets that are borderline in their content of this amino acid.

Balance studies of both the niacin and the tryptophane turnover of rats were made. Two experimental diets were used; the first of these consisted of 40 per cent of corn grits with 60 per cent of a virtually niacin free diet containing 15 per cent of casein, a diet shown by W. A. Krehl, L. J. Teply, and C. A. Elvehjem (*Science* **101,** *283 (1945); Nutrition Reviews* **3,** *267 (1945)*) to permit only poor growth in the rat. The second experimental diet contained the same ingredients as above, together with an added 1.5 mg. of niacin per 100 g. of diet. The tryptophane content of each diet was 118 mg. per 100 g.

Tryptophane balance studies were carried out by feeding male rats of the Sprague-Dawley strain on one of these two diets for a number of weeks, observing each week the food consumption, weight gain, and the urinary and fecal output of tryptophane. Additional rats were maintained on the two diets and 2 on each diet were killed each week for the assay of tryptophane in the entire body, so that a valid estimate could be made of the amount of tryptophane laid down in each gram of weight gain in any week of the experiment. The tryptophane content of the rat carcasses showed a remarkable uniformity. The mean tryptophane level from forty analyses was 1.70 mg. per gram of rat, with a range from 1.60 to 1.88 mg. To express the percentage utilization of the tryptophane of the diet, use was made of the formula: Utilization = $\frac{(\text{Tryptophane per gram of rat} \times \text{grams gained}) + \text{tryptophane in urine and feces}}{\text{Tryptophane ingested}} \times 100.$

In a first experiment 2 rats were maintained on each diet for a period of four weeks. Without niacin, the rats gained 6.0 g. per week with a food intake of 36 g., the tryptophane utilization was 30 per cent, and a food consumption of 6.3 g. was needed for each gram of gain in body weight. With niacin the food intake rose to 65 g., the utilization of tryptophane was 70 per cent, and 1 g. of weight gain was produced by 2.4 g. of food.

In a second experiment all the rats were fed on the diet without added niacin during a preliminary period of four weeks. Two of the rats continued to receive this diet for a further two weeks, while 2 others continued only for the fifth week, receiving the niacin-containing diet the sixth week. Data on food intake, weight increase, and excretion were collected. The latter 2 rats showed a poor food intake, poor utilization of tryptophane, and poor efficiency of food utilization during the fifth week, but a good appetite with remarkably good utilization of tryptophane and efficiency of food utilization during the sixth week. For each of these 2 rats during this period, the utilization of tryptophane was over 90 per cent and the food consumption per gram gain in body weight was approximately 1.8 g.

A similar balance sheet was drawn up at

the same time for niacin. Here again the body content of niacin was remarkably constant at about 33 micrograms per gram and at either level of niacin in the diet, the total of excreted niacin plus niacin laid down in tissues always exceeded the ingested niacin, as shown earlier by W. J. Dann (*J. Biol. Chem.* **141,** *803* (*1941*)). The actual synthesis of niacin must exceed considerably the figures reported by W. A. Krehl *et al.* (*loc. cit.*), since they failed to measure the excretion of niacin and its end products in the urine, and the results of F. Rosen, J. W. Huff, and W. A. Perlzweig (*J. Biol. Chem.* **163,** *343* (*1946*); *Nutrition Reviews* **4,** *334* (*1946*)) indicate that the greater part may be excreted in this way.

The authors point out that the biologic synthesis of niacin in the rat on a synthetic diet is decreased from 1100 to 280 micrograms per week by the addition of corn grits to the diet. The corn grits themselves added less than 100 micrograms of niacin to the diet per week. It is suggested that the tryptophane content of the basal diet is adequate for growth when with added niacin 70 per cent utilization is obtained, but that it is inadequate when without niacin, only 30 per cent utilization is obtained.

The known effect of the addition of tryptophane to corn-containing diets in improving growth may be due, it is suggested, to the action of the tryptophane in increasing niacin synthesis. The niacin could then act in two ways, by providing niacin for tissue growth and also by improving tryptophane utilization. Whether tryptophane acts by stimulating niacin synthesis in the rat, or whether it is a precursor of niacin remains unknown, although the latter is suggested by the work of F. Rosen, J. W. Huff, and W. A. Perlzweig (*loc. cit*). Their evidence is circumstantial, not direct. To provide direct proof that tryptophane is a niacin precursor seems to demand the use of "tagged" tryptophane. If tryptophane containing isotopic nitrogen in the indole ring could be given to rats on niacin free synthetic diets, the presence or absence of isotopic nitrogen in the synthesized niacin should solve the problem whether the tryptophane has acted as a direct precursor.

LYMPHOID ATROPHY AND ANTIBODY PRODUCTION IN PYRIDOXINE DEFICIENCY

The inanition and loss of weight which result from most dietary deficiencies are accompanied, as would be expected, by atrophy of many tissues. The degree of atrophy in a given tissue generally parallels the loss of body weight but the extent of change varies from tissue to tissue. Atrophy is such a common response to dietary restriction that careful study and measurements are necessary before such changes can be ascribed to specific effects.

H. C. Stoerk (*Proc. Soc. Exp. Biol. Med.* **62,** *90* (*1946*)) has studied the effects of a variety of dietary deficiencies on the thymus gland. The body weights and thymus weights of 8 week old rats maintained for four weeks under the following conditions were recorded: low protein, starvation, low calcium, vitamin B-complex deficiency, thiamine deficiency, riboflavin deficiency, pantothenic acid deficiency, and pyridoxine deficiency. In all but 2 cases there was an approximate linear relation between the amount of body weight deficit and thymus weight deficit. In pyridoxine deficiency, the amount of thymus weight deficit was much greater than expected from the loss of body weight, while in calcium deficiency an opposite effect was observed. Apparently, the calcium effect is not due to hypocalcemia because parathyroidectomized rats did not show such a relation.

The specific effect of pyridoxine deficiency in producing thymic atrophy was further

brought out by histologic studies. It was also shown that the injection of desoxypyridoxine (a compound having antipyridoxine effect) led to an accentuation of the atrophic effects. Since lymphoid tissue in general seemed also involved in this effect it is speculated that desoxypyridoxine may be of value in the treatment of tumors of the lymphoid tissues.

Since there have been recent observations which suggest an important relation between lymphocytes and antibody formation (W. E. Ehrich and T. N. Harris, *Science* **101,** *28* (*1945*); T. F. Dougherty, J. H. Chase, and A. White, *Proc. Soc. Exp. Biol. Med.* **57,** *295* (*1944*)), H. C. Stoerk and H. N. Eisen (*Proc. Soc. Exp. Biol. Med.* **62,** *88* (*1946*)) have made preliminary measurements on the effects of pyridoxine deficiency on the production of antibodies in the rat. Groups of approximately 10 rats each, 4 weeks of age, were placed on a pyridoxine deficient diet, a complete diet restricted in amount to duplicate the growth retardation of the deficient group, or a complete diet fed ad libitum. During the fifth week of the dietary regimen all animals were immunized against washed sheep erythrocytes. Five days after the last of three injections of 0.5 ml. of 5 per cent cells the hemagglutinin and hemolysin titers were determined by standard methods using twofold dilution steps. Although the scatter of the antibody values was great in both control groups, the differences between these and the deficient group were striking. Of the 9 pyridoxine deficient animals 6 showed no measurable agglutinins or hemolysins and 3 had only low titers. The average agglutinin titer was 1:04 and the hemolysin 1:13. In the control groups all of the 16 animals had measurable antibody titers. The average agglutinin titers were 1:64 and 1:412, and the average hemolysin titers were 1:36 and 1:68 for the pair-fed and ad libitum group, respectively. While it is assumed that the striking lymphoid atrophy present in these animals was responsible for the low antibody production, this effect could be an independent result of pyridoxine deficiency.

THE NUTRITIVE VALUES OF PROTEINS COMPARED WITH THEIR CONTENT OF ESSENTIAL AMINO ACIDS

The biological value of proteins is assumed to be dependent entirely on their content of essential amino acids, although few quantitative studies to prove this have been made. Such a study has been conducted by H. H. Mitchell and R. J. Block (*J. Biol. Chem.* **163,** *599* (*1946*)), using rats as experimental animals.

These authors have proposed that, until accurate values for the amino acid requirements of the growing rat are established, the best way to reveal the relation between the nutritional value of a protein and its known amino acid constitution is to calculate, per 16 g. of nitrogen, the percentage deficit for each amino acid, compared with the amino acid content of a protein mixture which is almost completely utilized by the growing rat. To illustrate this comparison, the figures may be quoted for a single protein from the extensive table, covering 28 proteins, which is compiled by the authors. It includes all those proteins for which satisfactory analyses have been made for all the essential amino acids. The composition of whole egg protein is taken as a standard which is completely utilized, and its percentage amino acid composition is stated as arginine 6.4, histidine 2.1, lysine 7.2, tyrosine 4.5, phenylalanine 6.3, tryptophane 1.5, cystine 2.4, methionine 4.1, cystine plus methionine 6.5, threonine 4.9, leucine 9.2, isoleucine 8.0, and valine 7.3. In view of the interrelationship of the sulfur-containing amino acids, the total content of cystine plus methionine is considered as a separate

item in addition to the content of each of these compounds separately.

For egg albumin, analysis showed that the content of arginine is 11 per cent less than that of whole egg protein (egg albumin contains 5.7 per cent of arginine against 6.4 per cent in whole egg protein), lysine 31 per cent less, tyrosine 7 per cent less, tryptophane 7 per cent less, threonine 22 per cent less, and isoleucine 11 per cent less. The other essential amino acids are present in as great or greater proportion in the albumin as in whole egg protein. From these figures the amino acid limiting the biological value of egg albumin appears to be lysine.

For the 28 proteins whose makeup is tabulated in this way, the biological value as reported in the literature (or for some proteins unreported in the literature, but determined in the Division of Animal Nutrition, University of Illinois) was compared with the percentage deficit of the limiting amino acid. Proteins arranged in order of increasing deficits were found to be roughly in order of decreasing biological value. The correlation coefficient between the maximum deficit and the biological value was calculated as $r = -0.86$, which is a high value. The regression equation relating the two variables was y (biological value) $= 102 - 0.634 \times$ (maximum deficit), which permits the approximate estimation of the biological value of a protein when its maximum percentage deficit of an essential amino acid is known.

The nutritive value of cereal mixtures can be considerably impaired by heating the mixtures, although no alteration could be demonstrated in their content of essential amino acids, as assayed chemically. As suggested by the authors, however, the differences in biological value before and after heating may be well within the limits of error of the amino acid analyses.

Some other exceptions occur in which the biological value of the protein does not agree with its rating by the deficit of the limiting amino acid. This may sometimes be due to inaccuracies in the data such as will be introduced by experimental errors and at other times to the fact that the biological values relate to the total nitrogen of the material assayed, while the amino acid analyses may not. The latter cause probably underlies the observation that animal tissues (muscle, liver, kidney, and heart) rank higher on the chemical scale than on the biologic scale, since these tissues contain considerable amounts of nonprotein nitrogenous substances.

Furthermore, the biological value is a function of the amino acids absorbed rather than the total amino acid content of the protein. Should the undigested residues be relatively high in a few essential amino acids, one would not expect the chemical and biologic scales to agree.

It is more difficult to explain those instances in which the protein is found to be better by biologic tests than by the chemical rating. If the fundamental thesis of this paper is sound, such data clearly indicate either errors in experimental data or that the levels of amino acids in egg protein are not minimum levels for the ideal protein, since any protein with a high biological value must have sufficient of the essential amino acids to give this biological value. F. J. Stare, D. M. Hegsted, J. M. McKibbin (*Ann. Rev. Biochem.* **14,** *431 (1945)*) pointed out previously that the levels of all amino acids in egg protein are not necessarily minimum levels. Thus it would appear that some attempt should be made to adjust the scale for the chemical rating to see if the few exceptional proteins, such as those of wheat and corn germ which are known to rate high biologically cannot be more adequately fitted in on the chemical rating.

However, the comparison of the chemical and biologic methods clearly supports the hypothesis that the biological value of a protein is due to its content of essential amino acids and is limited by the one essential amino acid which is present in the least amount relative to body requirements.

NUTRITIONAL AMBLYOPIA IN PRISONERS OF THE JAPANESE

Studies of Americans who had been prisoners of the Japanese have been described (*Nutrition Reviews* **4**, *208*, *253* (*1946*); **5**, *37*, *86* (*1947*)). Such reports have, in the main, dealt with a variety of manifestations of nutritional deficiency. Consequently the report by S. M. Bloom, E. H. Merz, and W. W. Taylor (*Am. J. Ophth.* **29**, *1248* (*1946*)) is of special interest for it deals specifically with the association of poor diet with visual impairment.

The study is based on observations in 33 soldiers captured in April and May 1942 on Bataan and Corregidor and liberated in early 1945. In the main, the diet of these men consisted of about 300 g. of polished rice plus a thin soup made of low grade vegetables and weeds. Negligible amounts of fruit were furnished occasionally. Meat was almost completely lacking.

During the first four months of imprisonment beriberi of both the wet and dry types, scurvy, pellagra, ariboflavinosis, and xerophthalmia were observed in these men. In addition, malaria, dysentery, and diphtheria affected many. Blurring of vision developed gradually after about four months on the diet described. This consisted of an intermittent fading, more marked in bright sunlight. After two to four weeks, pain behind the eyeball and a burning sensation in the eyes appeared in attacks during the late afternoon of bright sunny days or during the early evening. These attacks were accompanied by abnormal sensitivity to light and excessive secretion of tears. At first, no external evidences of abnormality were noted, at least by the crude methods available to the investigators. Later, the cornea was noted to be dry and roughened and superficial ulcers appeared. Then circumcorneal injection became evident. When a change of Japanese administration occurred and the diet was improved, particularly when evaporated milk and carabao liver became available in small amounts, the development of corneal ulcers was arrested. At this time, ophthalmoscopic examination became possible and this revealed pallor of the optic disc where the optic nerve enters the eye. Visual field examinations showed central and cecocentral blind spots with moderate constriction of the peripheral visual fields.

One of the investigators had an opportunity later to observe civilian internees at another camp, and in these individuals he noted similar disturbances in the eyes. For these persons, however, thiamine chloride became available and this was given in amounts of 25 mg. daily for two to three weeks. This dosage served to check the progress of the eye disease. Multiple vitamins were then given and improvement continued.

The soldiers originally observed were subsequently studied after liberation at an Eye Center in this country. They complained of decreased vision in both eyes, inability to see an object when using central vision, and burning and pain in the extremities. Neurologic examination showed peripheral neuritis and bilateral optic atrophy. The appearance of the men, however, was that of well developed and well nourished individuals. Examination of the eyes showed definite pallor of the temporal (lateral) portion of the optic discs and the macular area appeared normal in approximately 60 per cent. In the remainder it was mottled in appearance. Vision in each eye was very poor (20/200, not correctible). The peripheral fields showed mild generalized constriction but were not otherwise remarkable. The central fields showed a central scotoma (blind spot) of 5 to 8 degrees. These observations led to a diagnosis of optic atrophy secondary to nutritional deficiency. In addition, a few patients showed small corneal scars, the result of ulcers. A striking feature of the examination was the lack of correlation between

visual acuity and the extent of the optic atrophy.

It is not difficult to accept the conclusion of the investigators that malnutrition due to a deficient diet was the probable cause of the optic atrophy in these individuals. It is a little more difficult to feel certain that the deficiency was due to lack of thiamine. It is clear that this vitamin was lacking in the diet but it seems more difficult to accept this as the sole cause of the eye changes, since many other factors were lacking also. Thiamine therapy was tried in all the cases but the results were poor. The conclusion is drawn that the thiamine deficiency had existed so long that irreversible changes had taken place in the optic nerve. While this is certainly plausible, the possibility that the eye changes were unrelated to thiamine deficiency must be kept in mind. It should be pointed out that the patients showed ocular symptoms from vitamin A deficiency (xerophthalmia, including perforation of the eye in one case) as well as those eye changes which have been associated with thiamine deficiency. Furthermore, it is noteworthy that despite the probable vitamin A deficiency there was no complaint of night blindness.

FOLIC ACID AND PERNICIOUS ANEMIA

One question which immediately arose as a result of the observations that folic acid (Lactobacillus casei factor) in doses of 1 to 10 mg. produced remissions in pernicious anemia (*Nutrition Reviews* **4,** *102, 199 (1946)*) was whether this was the active substance (maturation factor) present in the liver extracts previously used successfully in the treatment of this disease. A paper by E. L. R. Stokstad and T. H. Jukes (*Proc. Soc. Exp. Biol. Med.* **62,** *112 (1946)*) answers this question in the negative. A concentrated parenteral liver extract containing 15 U.S.P. units of maturation factor per milliliter was practically devoid of free L. casei factor (1.0 mg. per milliliter) as tested by microbiologic methods. Furthermore, treatment of this liver extract with dried chicken pancreas, known to contain "vitamin Bc conjugase," failed to liberate free L. casei factor. This is evidence that the extract contains little of the conjugated folic acid of yeast (pteroylheptaglutamic acid) and, therefore, that this particular conjugate is excluded as the maturation factor. The correctness of the above two conclusions was confirmed by the failure of L. casei (folic acid) deficient chicks to respond to treatment with the liver extract, although they responded promptly and markedly to daily injections of 5 to 10 mg. of "liver" L. casei factor.

The nature of the active principle in liver extract and its relation to folic acid remain unknown. The first guess might be that the maturation factor is a particular conjugate of folic acid which unlike that isolated from yeast and liver is not easily hydrolyzable to components active for microorganisms or for use by folic acid deficient chicks. There are, however, other possibilities, such as the possibility that folic acid is not a component of the maturation factor at all but leads to remissions in pernicious anemia through correction of a defective biochemical mechanism required by the body for the synthesis of the maturation factor. Other possibilities of this sort also come to mind.

Further evidence that the maturation factor is not pteroylheptaglutamic acid (Bc conjugate) comes from the observations of A. D. Welch, R. W. Heinle, E. M. Nelson, and H. V. Nelson (*J. Biol. Chem.* **164,** *787 (1946)*) which show that this conjugate is ineffective in the treatment of pernicious anemia. One mg. orally of the heptaglutamate daily for twenty-one days gave no reticulocytosis, while an equivalent amount

(0.35 mg.) of pteroylglutamic acid (free L. casei factor) caused a definite response (reticulocytosis 4.2 per cent). This failure of the pernicious anemia patient to convert the heptaglutamate to free folic acid may, as pointed out by the authors, "account at least in part for the failure of pernicious anemia individuals to derive adequate amounts of folic acid from the diet." It is known that folic acid occurs in combined forms in natural sources and data are presented to show that the normal individual as contrasted with the patient with pernicious anemia can convert this conjugate to free folic acid. Perhaps this is a key to the etiology of pernicious anemia. When a normal subject (average urinary elimination, 2.5 mg. of L. casei factor daily) was given two daily intramuscular doses (2.5 mg.) of conjugate, 8.3 per cent was excreted in the urine as free L. casei factor (and none as the conjugate), while an equivalent amount of pteroylglutamic acid (free L. casei factor) similarly injected led to 16 per cent excretion. Daily injections of 2.5 mg. of the heptaglutamate for twelve days into a patient with pernicious anemia led to only 1 per cent excretion, while doses of 0.85 mg. of free L. casei factor led to elimination of 15 per cent of the dose.

Another observation of interest in connection with this hypothesis as to the cause of pernicious anemia is that the injection of liver extract (practically free of both L. casei factor and the hepta conjugate) nevertheless leads to an increase in the excretion of L. casei factor in the anemic patient.

Many aspects of the folic acid-liver extract-pernicious anemia picture remain obscure but it is evident that important revelations are only temporarily pending.

CHOLINE REQUIREMENTS AND TOXICITY

Choline is well established as an important dietary factor. It is required as a constituent of acetylcholine and certain of the phospholipids, and its role along with methionine, cystine, and creatine in the general metabolic process of transmethylation provides one of the most interesting aspects of recent studies in intermediary metabolism (*Nutrition Reviews* **2,** *358* (*1944*)). The absence of choline in the diet of the rat, rabbit, and dog leads to an excessive deposition of lipids in the liver and eventually to cirrhosis. A marked vascular congestion also occurs in the kidney of young rats which leads to hemorrhagic degeneration. The deficiency in chicks and turkeys results in perosis ("slipped tendon") and a decrease in egg production. All species studied show decreased growth rates as would be expected. The place of choline in the nutrition of man is not clear; however, beneficial effects have been attributed to its use in the treatment of human liver cirrhosis (*Ibid.* **3,** *158* (*1945*)).

Since the needs vary depending upon such factors as the protein (methionine and cystine) contents of the diet and body weight, and since only a limited number of studies have been reported, choline requirements can only be approximated.

Studies with the dog indicate that 0.1 to 0.3 per cent of the diet, or 10 to 100 mg. per kilogram per day of choline will prevent loss of weight and fatty cirrhotic liver (*Nutrition Reviews* **2,** *298* (*1944*); **3,** *124*, *261* (*1945*)). In the rat 120 to 200 mg. per kilogram per day (6 to 10 mg. per young rat) are reported to be necessary to prevent hemorrhagic degeneration of the kidney and the accumulation of excess lipoid in the liver (*Ibid.* **1,** *168* (*1943*)). This would be in the range of 0.1 to 0.2 per cent of the diet also. In the chick and young turkey a like level, 0.15 to 0.3 per cent of choline prevents

perosis and gives normal growth (*Ibid.* **2,** *50, 358 (1944)*). If it is assumed that the requirements for choline in man are of the same order of magnitude, i.e., 0.1 to 0.2 per cent of the diet, an adult would need from 1.5 to 3.0 g. of choline per day. Although the choline contents of foods have not been adequately explored, a rough estimation, using the limited analysis available (*Ibid.* **1,** *312 (1943)*), indicates that the daily diet of man may be expected to supply 1.5 to 4.0 g. of choline.

When man receives his dietary essentials from natural foods or when animal feeds are compounded from natural products, there is little likelihood that toxic levels of any of these factors will be reached even by the most distorted choice of foods and, therefore, until recently knowledge about the toxic levels of the various nutritive essentials was of academic interest only. However, with the wide use of concentrates and pure compounds for special therapeutic purposes and for the preparation of animal feeds, it becomes important for economic reasons to know whether the amounts in use exceed the needs for maximum benefits, and for public health reasons whether they exceed the levels of safety.

Some scattered data on the toxicity of choline for experimental animals are available and may be of use in fixing the approximate values for man. V. H. Melass, P. B. Pearson, and R. M. Sherwood (*Proc. Soc. Exp. Biol. Med.* **62,** *174 (1946)*) on the basis of experiments with chicks, estimate the level of choline which will lead to a decrease in rate of growth (mildly toxic) at between 1.0 and 2.0 per cent of the diet. One week old chicks maintained on choline levels of 1.0, 2.0, and 4.0 per cent of the diet gave the following responses: The addition of 1.0 per cent choline brought about a decrease in weight gain of 13 per cent while the 2.0 and 4.0 per cent levels gave 14 per cent and 24 per cent decreases, respectively. Statistical treatment of the data indicated that these were significant differences from the controls on the basal diet which contained 0.05 per cent choline. No toxic manifestations other than retarded growth were noted.

H. C. Hodge and M. R. Goldstein (*Proc. Soc. Exp. Biol. Med.* **51,** *281 (1942)*) determined the L. D. 50 dose for mice by intraperitoneal injection of a 2.0 per cent choline hydrochloride solution as 320 mg. per kilogram. The L. D. 50 dose by stomach tube for rats was found to be 6.7 g. per kilogram. Additional measurements were made with rats by M. W. Neuman and H. C. Hodge (*Ibid.* **58,** *87 (1945)*) in which the L. D. 50 dose for rats by stomach tube was found to be 3.4 to 6.1 g. per kilogram depending upon the concentration of solution used. Further studies by H. C. Hodge (*Ibid.* **58,** *212 (1945)*) in which rats were fed diets containing increasing levels of choline chloride showed that no effect on growth occurred at levels of 0.01 and 1.0 per cent choline chloride; however, levels of 2.7 per cent choline reduced growth by about 20 per cent. Five per cent choline decreased growth by 45 per cent and 10 per cent choline in the diet allowed no growth. Examination disclosed no consistent histopathologic changes which could be attributed to inclusion of choline in the food. Calculations from food consumption data show that the 1.0 per cent intake corresponded to 500 mg. per kilogram per day and the 2.7 per cent level to 1350 mg. per kilogram per day. In another experiment drinking water containing 1.0 per cent choline, consumed at a calculated level of 750 mg. per kilogram per day led to a definite decrease in rate of growth. Thus, it would appear that levels of choline above 1.0 to 2.0 per cent of the diet or in the range of 500 to 1000 mg. per kilogram per day will lead to minimum toxic effects as measured by decreased growth rate, while the L. D. 50 dose is probably in the neighborhood of 3.5 to 6.0 g. per kilogram per day. If the same degree of toxicity

is assumed for man, minimum effects would be expected somewhere between 15 to 70 g. of choline per day, while the L. D. 50 dose would be of the order of 200 to 400 g.

J. E. Davis and J. B. Gross (*Am. J. Physiol.* **144,** *444 (1945); Nutrition Reviews* **4,** *83 (1946)*) have described the production of hemolytic anemia in both dog and man by feeding simultaneously fat and choline. Under these conditions choline at levels of 10 mg. per kilogram per day for four days is claimed to give the effect. This is surprising inasmuch as this is a level of choline below that found necessary to prevent pathologic signs of deficiency. As pointed out previously (*Ibid.* **4,** *83 (1946)*) "it would be important to establish with comprehensive data whether the dietary essential, choline, when fed in various quantities with excess fat would consistently produce such effects."

Choline at a level of 6.0 g. per day has been used in the treatment of human liver cirrhosis with reported favorable effects (*Nutrition Reviews* **3,** *158 (1945)*). Thus, levels of choline (calculated) which might lead to minimum toxic effects (growth retardation) are not strikingly above those which have been used in treatment.

It becomes increasingly apparent that further knowledge concerning the toxic levels of dietary essentials is needed and that comprehensive data are required.

METABOLISM OF VITAMIN A AND CAROTENE IN DIABETES MELLITUS

In diabetes mellitus carotene is not used in a normal manner and the use of vitamin A itself is also defective (H. Brandaleone and E. P. Ralli, *Proc. Soc. Exp. Biol. Med.* **32,** *200 (1934);* W. Heymann, *J. Am. Med. Assn.* **106,** *2050 (1936)*). These suggestions were strengthened by a common observation of faulty visual adaptation among diabetic patients, which has been accepted frequently as evidence of either deficiency of vitamin A or an inability to use it normally (J. G. Brazer and A. C. Curtis, *Arch. Int. Med.* **65,** *90 (1940)*). It is true that most of this work was done before simple and dependable photocolorimetric technics for measurement of small amounts of vitamin A became available.

In some preliminary blood assays using these more simplified methods, M. S. Kimble, O. A. Germek, and E. L. Sevringhaus (*Am. J. Med. Sci.* **212,** *574 (1946)*) noted no tendency toward either a high level of carotene in the blood or a low level of vitamin A in cases of diabetes mellitus. Because of apparent discrepancies these investigators suggested that perhaps some extra diabetic factor was responsible for the abnormal levels which had been observed. They determined the levels of plasma carotene and vitamin A in 116 unselected cases of diabetes in which blood could be obtained before beginning hospital treatment and compared them with the levels of healthy control subjects of both sexes. The levels of carotene and vitamin A in the blood were measured by the method described by M. S. Kimble (*J. Lab. Clin. Med.* **24,** *1055 (1939)*). Males and females were considered separately because of the widely observed sex difference in levels of vitamin A in the blood (H. R. Getz and T. A. Koerner, *Am. J. Med. Sci.* **202,** *831 (1941)*).

Among diabetic patients, the levels of vitamin A in the plasma showed wide individual variability when compared with the normal controls. The most pronounced trend, however, was toward low levels of both vitamin and provitamin.

The effects of extra dietary vitamin, of defective absorption, and of hepatic disease on the level of vitamin A in the plasma are well known, and a careful survey of these histories revealed that fever, sepsis, perhaps acidosis, and a low fat diet may also influence

the levels of vitamin A. S. W. Clausen and A. B. McCoord (*J. Pediatrics* **13**, *635* (*1938*)) have pointed out that the levels of circulating carotene and vitamin A are lowered in the presence of sepsis and fever, and that although this deficiency in the blood may be acute, it need not be looked on as a sign of vitamin depletion. This deficiency occurs frequently in children and has been shown to take place in adult diabetic patients with fever. Likewise, levels of the vitamin in the blood were low among the diabetic patients who had chronic infections, which are not uncommon in this disease. Four of the patients who were admitted to the hospital in a state of coma had lowered levels of the vitamin in the plasma. These levels were similar to the levels in cases in which fever was present. In view of the profound disturbance of metabolism within the liver and kidneys in this condition it is not surprising that the amount of vitamin A in the blood is changed rather markedly. In 2 cases a sudden shift to a low fat diet seemed to cause a rapid reduction in levels of vitamin A in the plasma.

Factors which might cause an increase in the levels of vitamin A and carotene were also considered. These workers had already found that the level of vitamin A or carotene in the plasma of diabetics was not affected by the lipemia which occurred after a high fat meal. In cases of advanced renal disease associated with diabetes, however, there appeared to be an elevation in the levels of vitamin A and carotene. A satisfactory explanation for this is not yet complete. In another group of diabetic patients in a poor nutritional state a high level of carotene was noted. Although total starvation leads to exhaustion of the stores and to very low levels of both vitamin A and carotene in the blood, malnutrition in the presence of continued intake of foods containing carotene may result in accumulation of carotene.

The values for vitamin A and carotene found when the patients were admitted to the hospital were outside the normal range for the appropriate sex in 49 per cent of the 59 male patients and 47 per cent of the 57 female patients with diabetes mellitus. For the most part, the predominant type of deviation was a low level of vitamin A and carotene in the blood. This appeared to be most common among patients with infection and in the aged. However, in a few cases a trend toward high levels of carotene and low levels of vitamin A was noted. This irregularity has been assumed in the literature to be the result of an abnormality in the individual case of diabetes mellitus but in this group it seemed to be associated with malnutrition.

It appears, then, that there is no type of abnormality of the level of vitamin A or provitamin A in cases of diabetes mellitus which cannot be explained by consideration of the many abnormal states which may accompany or be a complication of diabetes mellitus.

BERIBERI AND EPIDEMIC DROPSY

From India there has been reported an epidemic dropsy almost indistinguishable clinically from the wet form of beriberi. The etiologic agent in this dropsy has been thought to be a toxic factor present in argemone oil (S. P. Mukherji, R. B. Lal, and K. B. L. Mathur, *Indian J. Med. Res.* **29**, *361* (*1941*)) or other foodstuffs in the diet. A report by E. R. Cullinan, A. Kekwick, A. S. Watts, and W. L. Titman (*Quart. J. Med.* **15**, *91* (*1946*)) is of interest in that it may indicate a relationship between this epidemic dropsy and beriberi. These workers describe an outbreak occurring in a portion of the East African forces which were garrisoned on an island town. The forces

were made up of British, African, and Mauritian troops. All of the troops were housed in good barracks and were stated to be living under excellent conditions. The climate was not unusual for this region. Suddenly on March 12, 1945, a group of men reported ill due to edema of the legs. During the next ten days all of the troops were either examined or questioned for the appearance of edema. An incidence of 38 per thousand individuals was found among the East African groups; no Europeans or Mauritians had the edema.

The chief complaint of the patients was swelling of the legs with accompanying feeling of tightness on walking. Some of the persons complained also of general fatigue. All of the subjects were afebrile while under observation. The general nutritional state of these individuals was better than that observed in a large group of the East African native soldiers who had previously been examined in a nutrition survey. Other evidences of malnutrition or deficiency states were consistently absent. There existed no venous or lymphatic obstruction to explain the edema. Clinical evidence of enlargement of the heart was not detected, although x-rays revealed right-sided dilatation of the heart in 4 out of 8 of the cases. The total serum proteins were not lowered. In fact, there was a slight increase above the normal for serum albumins in the 12 cases on which plasma protein determinations were reported. Peripheral neuritis as evidenced by "sluggish or absent deep reflexes" was observed in 18 cases. These observations suggested to the authors that they were dealing with a thiamine deficiency syndrome. Hence, 63 of the cases were divided into three groups and treated as follows: (1) 1 mg. of thiamine and 1 ounce of yeast daily, (2) 1 ounce of yeast, (3) "a small dose of ammonium chloride." The mean total duration of illness in the 29 patients treated with yeast and thiamine was 8.9 days, for the 18 patients treated with yeast alone 10.5 days, and for the 16 patients treated with ammonium chloride 14.7 days. It is not indicated whether this curative experiment was carried out prior to the institution of the unspecified dietary alterations or not. Dietary alterations, the nature of which is not indicated, were followed by a rapid decline in the development of new cases within the group.

In support of the thesis that this edema was a manifestation of thiamine deficiency the authors note that in November, 1944, rice was substituted as a staple food for maize meal previously issued to the soldiers. In February, 1945, the stock of beans in the garrison had been condemned, and, finally, during January and February, fresh vegetables were scarce. By February, 1945, the daily ration scale of the African troop diet contained 0.93 mg. of thiamine per 2835 calories. At the same time, the ration allotted to European troops contained 1.09 mg. of thiamine per 3891 calories and that of the Mauritians 0.49 mg. of thiamine per 2086 calories. Although the carbohydrate intake of the African troops was relatively higher than that of the Europeans and Mauritians, it does not appear that this level of thiamine intake by the East Africans would be expected to be associated with beriberi. Hence, the authors examined the case incidence of this epidemic edema in relation to the care and preparation of rice in the individual units. They concluded that "an impression is conveyed that the units paying attention to the rice had a lower incidence than those who did not."

Feeding experiments with pigeons employing the rice consumed by the individuals developing the dropsy demonstrated that the rice contained sufficient thiamine to protect the birds for a period of seventy days. When this same rice was cooked, however, 3 of the 4 birds fed the rice developed definite head retraction which responded to administration of thiamine. Examination of the rice itself indicated that 0.4 per cent of the weight was made up of a contamination with the spores and

sporangiophores of a variety of mucor. Also present were a trace of mouse feces and webbed chrysalises of moth.

The authors conclude that although the thiamine content of the rice appeared to be adequate to prevent beriberi, they believe that some other substance had developed within the rice which, during the process of cooking, destroyed the thiamine so that the cooked grain produced beriberi. They did not specifically relate this hypothetical thiamine-destroying substance to any of the extraneous material noted in the rice.

These investigators have not provided conclusive proof that epidemic dropsy and beriberi are both manifestations of thiamine deficiency. It is hoped that more precisely controlled tests of this hypothesis will be reported in the future. It is widely held that the thiamine requirement bears a relation to the quantity of carbohydrate in the diet. The suggestion contained in this paper, however, that some quantities of rice may be accompanied by a substance which renders thiamine more labile upon cooking, if confirmed, might add another explanation for the frequent association of thiamine deficiency manifestations with the eating of rice.

BIOSYNTHESIS OF ASCORBIC ACID

The ability of plants to synthesize ascorbic acid appears to be universal, and is the rule rather than the exception among animals, known exceptions being man, monkey, and the guinea pig. J. D. Guthrie (*Contrib. Boyce Thompson Inst.* **9,** *17 (1937)*) demonstrated the synthesis of ascorbic acid in potato tuber slices and found that this was dependent on aerobic conditions, and also that ethylene chlorhydrin stimulated the rate of synthesis. C. V. Smythe and C. G. King (*J. Biol. Chem.* **142,** *529 (1942)*) summarized some of the literature which claims to demonstrate synthesis in isolated animal tissues.

Several precursors in the biologic synthesis of ascorbic acid have been suggested. Both mannose and l-sorbose have been indicated, but their role in this synthesis has not been generally confirmed. Diketogulonic acid or 2-ketogulonic acid, although convertible to ascorbic acid by chemical means, are biologically inactive, hence do not appear to serve as precursors. Actually it appears that hexoses may not serve directly, but that three-carbon compounds may be concerned. The enhanced excretion of ascorbic acid and glucurinic acid are affected similarly by various factors, and available evidence favors synthesis of glucuronic acid from products of glucose metabolism rather than directly from glucose. By analogy, the same may be true for ascorbic acid synthesis, and there is increasing direct evidence for such a mechanism.

The demonstration by H. E. Longenecker, H. H. Fricke, and C. G. King (*J. Biol. Chem.* **135,** *497 (1940)*) that chloretone, chloral hydrate, and certain barbiturates increased the excretion of ascorbic acid in rats was extended by Smythe and King (*loc. cit.*) to studies of isolated tissues of rats. Liver and kidney slices of rats which previously received chloretone showed an increased synthesis of ascorbic acid. The addition of a mixture of hexosediphosphate, dl-glyceraldehyde, and pyruvate further increased the *in vitro* synthesis, while several other sugars and their derivatives were inactive in this regard. It was pointed out that a condensation of dihydroxyacetone phosphate (from hexosediphosphate) and l-glyceraldehyde with subsequent oxidation and ring closure is one possible mechanism for ascorbic acid synthesis. An apparent difficulty with such a mechanism is that ring closure or lactonization of mono- or diketogulonic acids has not been demonstrable

in biologic systems, although it is, of course, possible that other condensation products can readily undergo ring closure.

The report of S. C. Roy, S. K. Roy, and B. C. Guha (*Nature* **158,** *238 (1946)*) provides further evidence that ascorbic acid synthesis is dependent either on the products or at least the process of glucose metabolism. These investigators observed that the stimulating effect of chloretone on ascorbic acid excretion of the rat can be greatly suppressed if the animals are made deficient in either thiamine or riboflavin. The action of chloretone can be restored by correcting the respective deficiencies with thiamine or riboflavin feeding. Possible complicating factors such as inanition or abnormal destruction of ascorbic acid in these deficiencies seem to have been eliminated from consideration of the results.

The investigators refer to previous work which implicates narcotics in pyruvate oxidation, and have themselves confirmed the inhibitory action of chloretone on aerobic pyruvate oxidation. The role of thiamine and riboflavin in pyruvate metabolism is also recognized. It was concluded only that "if pyruvic acid is transformed into ascorbic acid in the narcotized rat, this process also requires the participation of thiamin and riboflavin." This could mean simply that these vitamins were involved as direct catalysts in the actual synthetic process, but it may be implied from the relations of both narcotic action and thiamine or riboflavin deficiency to pyruvate metabolism, that pyruvate or some product of its metabolism is a precursor for the synthesis.

It is difficult to formulate a single satisfactory hypothesis to account for the facts thus far reported. Actually the oxidation of pyruvate may serve merely as a specific source of energy for the synthesis, and the actual precursors be unrelated to products of pyruvate metabolism. The problem, therefore, remains unresolved as to the mechanism of ascorbic acid biosynthesis. If this process depends on the now fairly well recognized steps of intermediary carbohydrate metabolism, then there must still be some essential difference in the process in those species which are unable to synthesize ascorbic acid.

VARIATION IN HUMAN CALCIUM REQUIREMENT

The calcium requirement of adults, despite many investigations, is not well established, but it is recognized that there is considerable variation among subjects. In an investigation of the calcium metabolism and calcium requirements of 19 adult men, F. R. Steggerda and H. H. Mitchell (*J. Nutrition* **31,** *407 (1946)*) have considered particularly the variation among and within individuals, with reference to the ability to utilize ingested calcium, to the urinary and fecal excretion, and to the requirements for maintenance of calcium balance. The basal diet used was presumably adequate in all respects except calcium and contained an average of only 229 mg. of calcium per day. The subjects were maintained on a constant intake until analyses of urine and feces indicated that they were adjusted to this level of calcium intake. In succeeding metabolism periods, supplements of pasteurized whole milk, dried defatted milk, homogenized milk, a soft curd milk prepared by the process of base exchange, milk heated to 160°F. for thirty minutes, and milk with varied supplements of sodium alginate, potassium or sodium citrate, citric acid, and orange juice were tested to determine the utilization of the calcium from these sources. Calcium citrate and calcium gluconate were also tested as sources of calcium. During the periods when milk supplements were given, the intake of the basal diet was reduced to

equalize the energy intakes during the basal and test periods.

During the basal periods when each man ingested an average of only 229 mg. of calcium per day, the average calcium balance was −125 mg. per day, with a standard deviation of 57.2 mg. per day. A distinct variation among subjects in the urinary and fecal excretion was observed, with no inverse correlation between calcium balance and fecal excretion. For example, in 12 of the 19 subjects, the fecal calcium exceeded the intake, and for 17 subjects the fecal calcium excretion exceeded the urinary calcium. For 8 subjects, urinary calcium averaged less than 100 mg. daily per man, but for 6 subjects it was equal to or exceeded fecal calcium.

The supplements of calcium were fed at such levels that the total daily intake of calcium averaged 545 mg. per man. Statistical analyses of the data obtained on the utilization of calcium during the test periods indicated that the calcium utilization was essentially the same irrespective of the source which supplied the calcium. The average utilization of calcium was 31.6 per cent with a standard deviation of 7.36 per cent.

The indicated calcium requirements averaged 644 mg. per man per day, with a standard deviation of 181 mg. The mean requirement of calcium, expressed by different standards of measurement, was calculated to be 9.21 mg. of calcium per kilogram of body weight, or 343 mg. of calcium per square meter of body surface, or 0.366 mg. per basal calory. The coefficients of variation were about 28 per cent whether the requirement values were expressed per subject, unit of weight, basal calory, or of surface. This indicated not only that the variable of body size was not eliminated by these measurements, but that one or more extensive variables in calcium metabolism had a greater influence than body size or basal metabolic rate. Steggerda and Mitchell concluded that 10 mg. of calcium per kilogram of body weight per day is a good average value for adult men subsisting on diets containing dairy products to furnish one-half to two thirds of their calcium.

F. R. Steggerda and H. H. Mitchell (*J. Nutrition* **31,** *423 (1946)*) have also investigated the effect of the concentration of the citrate ion, which varies greatly in common diets, upon the calcium metabolism of human adult subjects. The subjects in these experiments were 17 of the 19 men in the previous investigation. The citrate content of the basal diet or the basal diet plus milk was modified by the addition of citric acid or its salts or by orange juice. Tests on the availability of the calcium in calcium citrate indicated that this source was as good as the calcium in calcium gluconate, which had been shown to be as available as the calcium in milk. When a daily supplement of 9 g. of potassium citrate or 4.5 g. each of potassium citrate and of citric acid was added to the basal low calcium diet, no consistent effect upon the calcium balance was observed. When a supplement of sufficient milk to induce a slight negative calcium balance or at most a slight positive calcium balance was added to the basal low calcium diet, a supplement of either 9 g. of sodium citrate or an addition of 0.27 per cent citric acid to the milk supplement did not affect the utilization of calcium in the milk during the twenty day period of administration. However when the sodium citrate or the citric acid supplements were discontinued, the calcium metabolism during subsequent periods was altered markedly in some subjects. The utilization of the calcium in milk was not consistently modified by the ingestion of orange juice in considerable quantities during the test period. However after discontinuation of the orange juice supplement, there was generally a disturbance in calcium metabolism. The changes observed after the sodium citrate and orange juice supplements were stopped, were characterized generally by a phase of increased calcium retention followed or preceded or both by

a period of increased excretion. The degree of these changes was definitely beyond the limits of variation produced by uncontrollable experimental factors and individual differences and seemed to be related to the prior administration of the citrate ion. After citrate administration, it was not uncommon for the calcium content of the feces of subjects maintained on the low calcium diet to be twice as great as the calcium consumption.

L. J. Schroeder, W. M. Cahill, and A. H. Smith (*J. Nutrition* **32,** *413 (1946)*) have studied the utilization of calcium in soybean products. The soybeans were prepared by autoclaving the whole beans with 2 parts of water at 15 pounds pressure for one hour. Soybean "milk" was usually diluted with water and chilled before serving. The experimental approach of Steggerda and Mitchell was followed. The utilization of calcium was found to be 29.1 per cent for evaporated milk, 23.7 per cent for calcium sulfate, 22.6 per cent for soybean "milk," and 10.4 per cent for whole cooked soybeans. Whole soybeans were a poor source even though the amount of calcium present was high. The authors speculated that the interference in calcium utilization by whole cooked soybeans might be due to the relatively large amount of roughage in the whole soybeans which had been removed in the process of preparation of soybean "milk." Considerable individual variation was observed in the values obtained from the 13 male subjects in this series of experiments, corroborating the studies of Steggerda and Mitchell.

These data indicate some of the various factors which influence calcium metabolism. In view of these factors, the average value of the calcium requirement of adult men suggested by Steggerda and Mitchell on the basis of their experiments is of significance in planning diets to furnish adequate amounts of calcium from sources whose utilization coefficient is known. However as Steggerda and Mitchell point out, such an average value for the calcium requirement is of little value in assessing the status of calcium nutrition in a community without the aid of balance studies. Several reports from dietary surveys indicate calcium intakes considerably below the average value of 10 mg. per kilogram of body weight. Either calcium deficiency is one of the most frequent nutritional deficiencies, or there are calcium sparing mechanisms which result in a greatly increased utilization of the small amounts of calcium present in the diet.

LONGITUDINAL STUDIES OF GROWTH, DEVELOPMENT, AND NUTRITURE

The longitudinal type of study is one in which data are collected on a given group of individuals over a time span and is in contrast to the horizontal study which evaluates the status of a group of individuals at a given instant in time. The usual nutrition survey is an example of this latter study. The measurements obtained on a population during the course of such a survey are useful, but obviously cannot be extrapolated in time. It becomes desirable, therefore, to have longitudinal observations on many persons in an effort to test whether correlations exist between certain minor differences in attributes over a period of time and the quality of health of the individuals. The value of this approach in the definition of desirable standards for nutrition studies has been implied in several reviews in this journal (*Nutrition Reviews* **3,** *114 (1945)*; **4,** *175, 247 (1946)*).

In groups of children in which one would expect to find signs of clinical malnutrition, specific lesions usually attributable to vitamin deficiency states are often surprisingly rare. The one manifestation of undernutrition most often noted in such groups is underdevelopment. Thus, among Italian, French, and Greek children, during periods of food

scarcity in recent years, the observation commented upon most frequently by investigators has been the retarded growth and development of these children (*Nutrition Reviews* **5,** *28, 104 (1947)*). Growth, then, seems to be a sensitive index of the nutritional state in human beings as well as in experimental animals. In 1943 H. C. Stuart (*Am. J. Dis. Child.* **65,** *320 (1943)*) noted that "Studies of human nutrition have been confined largely to assessment of the health of specific tissues and of the levels in the blood of various nutritional substances. Controlled experiments usually deal with the effects of varying one factor in the diet on one or a few physical or biochemical attributes. The period of test is usually, and of necessity, relatively short in respect to the whole cycle of growth and often too short for measuring the increments of growth satisfactorily." He further noted that it was impossible to define with any exactness the rate or pattern of growth which is most satisfactory from the standpoint of health and physical fitness. However, he stressed that there was reason to believe that the rate of growth during infancy and childhood could be modified by diet even under circumstances of dietary intakes which led to no other demonstrable abnormalities. Stuart urged that in nutrition research the growth of children be measured in so far as practical in connection with studies of the diet and other appraisals of nutritional status.

One of the major difficulties in carrying on nutritional investigations involving these longitudinal studies of growth is that of choosing from the multitude of proposed measurements those which would give useful information regarding the growth and development of children. The usefulness of some of the height-weight measurements, particularly the application of the Wetzel Grid, has been previously considered (*Nutrition Reviews* **1,** *250 (1943)*). The selection of useful body measurements for a school health program has been considered by H. C. Stuart and H. V. Meredith (*Am. J. Pub. Health* **36,** *1365 (1946)*), and this discussion should prove helpful to persons interested in longitudinal studies of growth in relation to nutritional investigations. In discussing the selection of useful measurements for a school program, these authors note the importance of keeping the list of recommended measurements as short as possible and of avoiding those procedures which are too time-consuming and difficult to carry out. They point out, however, that height-weight measurements alone do not give a complete picture of growth; rather, that body weight fails to differentiate between the amount of protoplasmic tissue and the amount of fat or water stored in these tissues. Furthermore, it does not distinguish between the more inert tissues, such as bone, and the more active protoplasmic tissues, such as muscle. They recommend as a minimum routine of anthropometry seven determinations as follows: body weight, standing height, hip width, chest circumference, leg girth, and careful subjective ratings of the thickness of two selected folds of skin and subcutaneous tissue. The methods for securing these data are detailed in the second portion of this paper. It is emphasized that other anthropometric methods are useful and may have certain advantages, but that these seven procedures are simple enough to permit their routine employment in examinations, such as school health studies.

These authors warn that the interpretation placed on such measurements, due to faulty choice or use of norms, can render a program of measurement of no avail or may lead to making recommendations which are harmful. They recommend that the interpretation be based upon the position of each measurement within the distribution of measurements rather than upon the relation of a measurement to the mean or average alone. "This tends to counteract the erroneous inference that the average is the standard all should attain, or that a certain percentage deviation from the average separates the normal and satisfactory from the abnormal or unsatisfactory."

As an example of the consideration of measurements in terms of distributions, the authors present a series of selected percentiles for height, weight, hip width, chest circumference, and leg girth. These tables give, for half year intervals, the measurements below which 10, 25, 50, 75, and 90 per cent of the children in the given age-sex group fell. By use of these tables one can determine, for example, whether by a particular measurement the child falls in the upper 50 to 75 per cent of the group, or in the lower 10 to 25 per cent. The use of percentiles rather than the standard deviation for placement of individuals within the distributions is advisable here because of the skewness of some of the distributions. These measurements are so easily made that they could well be included in most programs of nutrition investigation, even of the more rapid survey types.

H. C. Stuart (*New Engl. J. Med.* **234,** *666, 693, 732 (1946)*) has also reviewed the results of a large group of studies which have attempted to define normal growth and development during adolescence. This review should be of value for nutrition investigators in their effort to select standards of growth and development during this period. Although these studies are too extensive to discuss in detail here, it is pertinent to consider a few of the generalizations made in this review. One of these is that pronounced differences may exist between healthy children of the same chronologic age. The age at which the so-called "adolescent spurt" of growth occurs may vary over a period of five years or more. Similarly, there is no fixed chronologic age at which other signs of maturity appear. When chronologic age is ignored and the age of either initial growth acceleration or maximal growth is substituted, there is much greater uniformity between the various attributes of the children. Likewise, if the growth data on a group of girls with the menarcheal ages of all the groups superimposed is plotted, the curves are much more nearly alike in placement than if the development is merely plotted with chronologic age. In other words, the pattern of growth for adolescent children is similar when the physiologic age is considered. Several years ago, D. Wiehl (*Milbank Memorial Fund Quart.* **19,** *45 (1941)*) and D. Wiehl and H. D. Kruse (*Ibid.* **19,** *241 (see footnote p. 245) (1941)*) pointed out a similar relationship between hemoglobin concentration of adolescent children and their physiologic age.

Another generalization of interest in this review is the evidence from several studies which demonstrates that adolescents are larger today than similarly aged adolescents of a half century ago. Stuart notes that "The causes for these evidences of increasing size with time call for further study, but they are doubtless related to differences in the incidence of illnesses in early life, to dietary habits, to habits of activity and to other more obscure factors generally referred to as socio-economic."

It will only be through the most critical longitudinal studies of the relationship of nutrition to growth and health as presented in the papers herein reviewed that one will be able to sort out the role of nutrition in the complex of factors influencing health in childhood. N. B. Capon (*Arch. Dis. Childhood* **20,** *52 (1945)*) refers to nine such factors as "foundations of good health in childhood": (1) healthiness of the parents, (2) the character of the child's birth and the state of his health during the neonatal period, (3) a suitable diet, (4) healthy hygiene and management, (5) a safe, comfortable, and peaceful home, (6) training in the formation of good and regular habits, (7) the stimulus of good example, (8) opportunities for self-expression and development (physical, intellectual, and moral), and (9) freedom from infection and disease. It may be questioned that in practical child health studies investigations can ever be designed to evaluate separately any one of these nine influences.

NOTES

Letter to the Editor

Dear Sir:

In the October, 1946 issue (*Nutrition Reviews* **4,** *298* (*1946*)) there is a review of the paper by V. A. Beal, B. S. Burke, and H. C. Stuart which appeared in the *Am. J. Dis. Child.* **70,** *214* (*1945*)) dealing with the caloric intakes of children between the ages of 1 and 10 years.

This review states "these estimates of intake were calculated from one-day dietary records kept by the parent . . ." and again "Inasmuch as the dietary records were kept for but a single day at a time there may have occurred a tendency for the child to overeat . . . ." I would like to call your attention to the fact that these estimates were based upon three consecutive days' intakes kept by them plus detailed nutrition histories taken at each clinic visit. The results from the two sources were compared and the figure used at each age is the mean derived from the nutrition history and the average of the three consecutive days reported by the parents. It is most important to appreciate this, since a one day intake is of doubtful value, as the reviewer states, and the error makes a careful piece of work appear to be very careless. Contrary to the opinion of the reviewer there is also little chance where over 5000 intakes were studied that any tendency to overestimate the intake on the part of some would not be compensated for by underestimation on the part of others. Therefore, the mean value would be representative.

May I call your attention to a recent comment made in regard to these same caloric estimates by N. C. Wetzel (*J. Pediatrics* **29,** *444* (*1946*) *footnote to Energy Relations*) "The results of Stuart and his co-workers reporting an elaborate fifteen-year study at the Harvard School of Public Health covering 5,916 diet records of 269 children from 1 to 10 years old have appeared. When these are compared with the corresponding Grid estimates of fuel requirements, the agreement is statistically exact. This verification of the level-calorie relations in the Grid technique is all the more remarkable because of the entirely independent origin of the results."

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The Copenhagen Conference of FAO

As an outgrowth of the food conference at Hot Springs, Virginia, in 1943 (*Nutrition Reviews* **1,** *321* (*1943*)), the United Nations Food and Agriculture Organization (FAO) assumed a permanent form (*Ibid.* **4,** *8* (*1946*)). The first conference of FAO was held at Quebec City, Canada, in 1945; the second conference was held in Copenhagen, Denmark, September 2 to 13, 1946 (*FAO Information Bull.* **1,** *no. 6, Oct. 4, 1946*). Thirty-three member nations were represented at the conference by voting delegates, 8 by observers. Seven nonmember countries and 10 intergovernment organizations sent observers.

The central theme of the Copenhagen Conference was the organization of a permanent method of dealing with long-term problems. In Copenhagen it was recognized that when the current emergency ends the major world food problem will be the existence of unmarketable surpluses of food in the world while hundreds of millions of persons remain underfed.

In consideration of nutrition education it was stated that "The lead in the study and attack on nutrition problems in different parts of the world must be taken by a 'core' of specialized nutrition experts trained in

universities or other scientific institutions. To accelerate progress, a systematic instruction in the theoretical and practical aspects of food and nutrition must also be given to administrators, agricultural experts, food technologists teachers, nurses, and social workers. The Committee was unanimous in its opinion that the teaching of nutrition in various curricula, especially in medicine, is unsatisfactory. It considered that university authorities and others concerned with higher education should accord nutrition a place in their curricula in keeping with its importance."

Biologic Activity of 3-Methyl-Ascorbic Acid

The salts of certain metallic hydroxides, some organic amines, and some compounds structurally related to ascorbic acid (d-arabo-ascorbic acid, 6-desoxy l-ascorbic acid, l-rhammoascorbic acid, l-glucoascorbic acid, l-fucoascorbic acid, d-glucohepto ascorbic acid, and l-scorbamic acid) have been claimed to have at least some, usually only a small fraction, of the antiscorbutic activity of ascorbic acid (H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," *p. 313, Interscience Publishers, Inc., New York (1942)*). The claim of N. Bezssonoff and R. Sacrez (*Compt. rend. Soc. de biol.* **124,** *356 (1937)*) that 3-methyl l-ascorbic acid possesses a degree of antiscorbutic activity has been reinvestigated by C. S. Vestling and M. C. Rebstock (*J. Biol. Chem.* **164,** *631 (1946)*).

Daily supplements of 20 mg. of pure crystalline 3-methyl-ascorbic acid, given intraperitoneally or by mouth to guinea pigs, appear to provide the equivalent of less than 2 mg. of l-ascorbic acid. No evidence was obtained that this activity was due to a hydrolysis to ascorbic acid.

The biologic function of ascorbic acid is evidently dependent on more than its reducing properties; in fact, studies of the past few years indicate that slight modifications in the chemical structure of vitamins may lead not to partial replacement of the vitamin function, but actually to antagonism with the natural compounds.

A New Synthesis of Provitamin D_3

The method heretofore in general use for the preparation of 7-dehydrocholesterol, which on irradiation yields vitamin D_3, is that introduced over a decade ago by A. Windaus, R. Lettré, and F. Schenck (*Ann. d. chemie* **520,** *98 (1935)*). In this method the over-all yield of the provitamin from cholesterol is about 4 per cent only.

H. B. Henbest and co-workers (*Nature* **158,** *169 (1946)*) have given a preliminary account of a new method of preparation of 7-dehydrocholesterol which gives improved yields. In this procedure cholesteryl acetate is treated with N-bromosuccinimide to yield beta-7 bromo-cholesteryl acetate. This compound on heating with diethylaniline loses hydrobromic acid to give 7-dehydrocholesteryl acetate, which can be easily hydrolyzed to the parent 7-dehydrocholesterol and purified.

Under suitable conditions, cholesterol can be converted by these reactions into 7-dehydrocholesterol with a yield of 30 per cent. The use of this method may have far reaching effects on the vitamin D industry. It is possible that it will reduce the cost of vitamin D_3 so that it will tend to replace vitamin D_2, on the basis of preference for the use of the natural form of the vitamin as it occurs in fish liver oils.

Nutrition Bulletin

The Ministry of Food, His Majesty's Stationery Office, York House, Kingsway, London, W.C.2, publishes a monthly bulletin entitled "*Food and Nutrition*" (Price: four shillings) which should be of interest to many of the readers of NUTRITION REVIEWS. The January, 1947 issue deals particularly with facts about milk.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

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